gastrointestinal tract. Targeted injections were directed to neural and gut precursors using an antisense morpholino oligonucleotide. In embryos targeted for the neural crest, loss of anterior structures was observed, and neural crest marker expression was down regulated. In embryos targeted for the foregut, organ budding was impaired. Because C3 is a known chemoattractant involved in blood cell migration, development of the ventral blood islands was also examined in the knockdown embryos and found to be compromised. In summary, we report the first functional data supporting a patterning role for the vertebrate complement cascade. Putative mechanisms include signaling through C3aR, a G-protein-coupled receptor.

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Program/Abstract # 421
Rho GTPase signaling directs the late stage morphogenesis of the *Xenopus* digestive system

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The primitive gut tube undergoes dramatic narrowing and elongation to shape the functional anatomy of the digestive system, but the morphogenetic mechanisms underlying these topological transformations are unknown. A small molecule screen in *Xenopus* embryos revealed that Rho kinase inhibitors induce severe defects in gut tube elongation. These chemical genetic results were molecularly validated by showing that Rho family GTPases are expressed in the developing gut tube, and that the gut deformities induced by chemical inhibition of Rho kinase are phenocopied by injecting mRNA encoding an inhibitory version of *Xenopus Rho*. Treatments with the potent Rho kinase inhibitor, Rockout, perturb gut elongation in a concentration-dependent manner, and reveal that Rho GTPase signaling is required just prior to and during the stages of overt gut lengthening. In situ hybridization analyses confirm that inhibition of Rho signaling does not alter gut patterning; however, immunohistochemical studies show that Rockout treatment reduces endoderm proliferation, perturbs intercellular adhesion, and disrupts the acquisition of cell polarity necessary for intestinal epithelial morphogenesis. These results show that Rho GTPases play similar roles in the morphogenesis of the endoderm as they do in other tissues, and suggest intriguing analogies between the elongation and narrowing of the gut tube and the convergent extension movements of gastrulation. This study underscores the utility of small molecule screening in *Xenopus* embryos for elucidating novel mechanisms of late stage morphogenesis.

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Program/Abstract # 422
Expression of EphA9, a gene important for proper cell migration during avian gastrulation, is regulated by FGF signaling and a GSK3-dependent but Wnt-independent pathway

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Gastrulation is a critical early event in development that leads to formation of the primary germ layers (ectoderm, mesoderm and endoderm). In amniotes, gastrulation is characterized by extensive cell rearrangements, including epithelial–mesenchymal transition (EMT) in the primitive streak. To investigate how EMT is initiated during gastrulation we focused on genes upregulated in the chick epiblast just lateral to the primitive streak ("preingression zone"), then downregulated as cells undergo EMT. *EphA9* is an RTK expressed specifically in the preingression zone. We performed experiments to investigate *EphA9* function and regulation. Expression of a dominant active *EphA9* receptor led to an accumulation of cells in the epiblast and primitive streak with a reduced number of cells in the mesoderm, indicating that *EphA9* downregulation is required for proper cell migration. Treatment of embryos with the FGFR1 inhibitor SU5402 or the ERK inhibitor U0126 led to downregulation of *EphA9*, while exposure to GSK3 inhibitors LiCl or SB415286 led to upregulation and lateral expansion of *EphA9* expression in the epiblast. While this latter result implicates canonical Wnt signaling, forced expression of dominant active β-catenin downregulated *EphA9* expression. These findings lead to a model where *EphA9* expression in the epiblast is dependent on FGF signaling via FGFR1 and ERK, and a GSK3-dependent pathway independent of canonical Wnt signaling. Downregulation of *EphA9* during EMT is likely regulated by canonical Wnt signaling.

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Program/Abstract # 423
The role of FGF signalling in the formation of the primitive streak

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We study the process of streak formation in the chick embryo. We have previously characterised the movements of the cells in the epiblast that precede the formation of the streak and shown that they involve large scale co-ordinated cell movements, forming two counter-rotating cells flows that merge at the site of streak formation. These large scale cell movements bring the mesoderm, that is induced by signals form the posterior marginal zone in the epiblast overlying Koller's Sickle, into the midline of the embryo (Chuai et al., 2006). Several FGFs are expressed in the mesoderm and

forming primitive streak, notably FGF8. Inhibition of FGF signalling, through depletion of FGFs or inhibition of FGF8 expression results in a failure of mesoderm differentiation and streak formation, while increased FGF signalling stimulates axial mesoderm formation, but inhibits streak formation. In order to dissect the signalling pathways to mesoderm differentiation and movement we have inhibited several downstream signalling pathways. We show that FGF-mediated mesoderm induction is dependent on signalling through both the ERK/MAP kinase and the PI3 kinase pathway. Inhibition of either of these pathways results in inhibition of mesoderm formation as well as streak formation. However, overexpression of Sprouty2, a negative regulator of FGF receptor signalling, does not inhibit mesoderm formation, but does effectively inhibit the cell movements associated with streak formation. The underlying mechanisms are now under further investigation.

Reference

Chuai, M., et al., 2006. Cell movement during chick primitive streak formation. Dev. Biol. 296, 137–149.

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Program/Abstract # 424
Visualization of the epithelial-to-mesenchymal transition of individual trunk neural crest cells

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During avian neural morphogenesis at in the trunk at E2, neural crest cells undergo an epithelial-to-mesenchymal transition (EMT) in order to leave the dorsal region of the neural tube. To accomplish this EMT, neural crest cells must lose adhesion to the apical lumen of the neural tube and leave the neural tube at its basal surface. This developmental event has long fascinated biologists, yet it has not been observed directly. This leaves certain questions about the avian trunk EMT unanswered. Are neural crest cells required to lose apical attachments before they leave the neural tube? Is an asymmetric mitosis responsible for the EMT of trunk neural crest cells? We have imaged individual dorsal neural tube cells labeled with membrane-localized EGFP using time-lapse, laser-scanning confocal microscopy. No instances of asymmetric mitosis among neural tube cells have been observed. In some cases, neural crest cells leave behind small pieces of themselves as their trailing process breaks away from the apical surface. Some neural crest cells exit the neural tube at the position of their basal process. However, other neural crest cells form a new process and leave the neural tube at an entirely different location. While we verify that neural crest cells do eventually lose contact with the apical lumen of the neural tube, our observations suggest that the activation of cell motility may be more important in the EMT of trunk neural crest cells than the loss of cell–cell adhesion.

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Program/Abstract # 425
Molecular and cellular mechanisms of cranial skeleton development

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While the mechanisms of skeletal development in the trunk of an embryo are well understood, the molecular program that directs analogous process in the head remains largely uncharacterized. In the axial skeleton of the trunk, cartilaginous elements originate from the sclerotome. The situation in the head is more complex as the cranial skeleton receives contributions from cephalic paraxial mesoderm, neural crest and occipital somites. The aim of this investigation is to elucidate the molecular and cellular interactions that result in the induction of the cranial base using the developing chick embryo as an experimental system. Our results show that, like craniofacial myogenesis, development of the cranial base is delayed relative to the trunk. Yet, whilst Shh, the principal inducer of cartilage differentiation in the trunk, is expressed along the entire rostro-caudal axis in the notochord and in the neural tube, analysis of the expression of the Shh transcriptional targets, Ptc1 and -2, indicates that Shh signalling is not active in the early cranial mesenchyme. This suggests that Shh activity is antagonised by, as yet, unidentified signals. In the chick embryo, the chondrocranium starts to differentiate at stage 25. Recent data in zebrafish has indicated a role for Shh in the development of the chondrocranium. This suggests that although Shh signalling is initially blocked it may be later required for development of the cranial base. Analysis of Ptc1 and -2 expression indicates that this is very likely. Functional studies are in progress to analyse the role of Shh and to identify the tissue interactions required for chondrogenic differentiation.

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Program/Abstract # 426
The roles of tenascin-W in osteogenesis

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Tenascins are a family of large glycoproteins found in the extracellular matrix (ECM). We are characterizing the final